**Original DNA Sequence Result:**

NNNNNNNNNNNTATNGNATACTCAAGCTATGCATCCAACGCGTTGGGAGCTCTCCCATATGGTCGACCTGCAGGCGGCCGCACTAGTGATTTTGATTGATTGAAGGAGAAATATCATGAGTGTGATCGCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAATGGACACTACTTTGAGGTCGAAGGCGATGGAAAAGGAAAGCCTTACGAGGGGGAGCAGACGGTAAAGCTCACTGTCACCAAGGGTGGACCTCTGCCATTTGCTTGGGATATTTTATCACCACTGTCTCAATACGGAAGCATACCATTCACCAAGTACCCTGAAGACATCCCTGATTATGTAAAGCAGTCATTCCCTGAGGGATATACATGGGAGAGGATCATGAACTTTGAAGATGGTGCAGTGTGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTACAATGTCAAAATCTCTGGTGTGAACTTTCCTCCCAATGGACCTGTTATGCAGAAGAAGACACAGGGCTGGGAACCCAACACTGAGCGTCTCTTTGCACGAGATGGAATGCTGATAGGAAACAACTTTATGGCTCTGAAGTTGGAAGGAGGTGGTTACTATTTGTGTGAATTCAAATCTACTTACAAGGCAAAGAAGCCTGTGAGGATGCCAGGGTATCACTATGTTGACCGCAAACTGGATGTAACCAGTCACAACAAGGATTACACATTTGTTGAGCAGTGTGAAATATCCATTGCACGCCACTCTTTGCTCGGTCATCACCATCACCATCACTAAAATCCCGCGGCCATGGCGGCCGGGAGCATGCGACGTCGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAANAGGCCCGCACCGATCGCCCTTCCCACAGTTGCGCAGCCTGATGGCNATGGACGCNNCCTGNANCGNCGCATTAGCNCGGCNGGNNNGNGNNCNCGCAGCGNGACNCTACANTTNCNGCNNCNANCNNNNNCNTTCNNTTNTNCNNNTNNNNNNNNNNNNNNNTTNCCGNNAGNTNNAANNGGGGNNNNNNNNNATNNGNTNNNNNNNNNNCCAAANTGNNNGNNNNGNNNNNNNNGNNNNNNNNNNNNNNNNNNNNNNNGANCNNNNNNNNNNNNANNNNNNNNNNNCNNNNNAANNNNNCCNNTNNN

Bases: 1,286

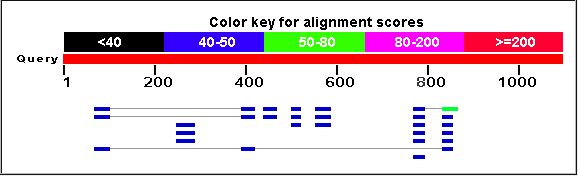
**After removing ends from the Top and the End of the Sequence:**

We removed 11 bp from the top and we removed the end by looking for the first 5 N’s in a row and removing everything after that.

TATNGNATACTCAAGCTATGCATCCAACGCGTTGGGAGCTCTCCCATATGGTCGACCTGCAGGCGGCCGCACTAGTGATTTTGATTGATTGAAGGAGAAATATCATGAGTGTGATCGCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAATGGACACTACTTTGAGGTCGAAGGCGATGGAAAAGGAAAGCCTTACGAGGGGGAGCAGACGGTAAAGCTCACTGTCACCAAGGGTGGACCTCTGCCATTTGCTTGGGATATTTTATCACCACTGTCTCAATACGGAAGCATACCATTCACCAAGTACCCTGAAGACATCCCTGATTATGTAAAGCAGTCATTCCCTGAGGGATATACATGGGAGAGGATCATGAACTTTGAAGATGGTGCAGTGTGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTACAATGTCAAAATCTCTGGTGTGAACTTTCCTCCCAATGGACCTGTTATGCAGAAGAAGACACAGGGCTGGGAACCCAACACTGAGCGTCTCTTTGCACGAGATGGAATGCTGATAGGAAACAACTTTATGGCTCTGAAGTTGGAAGGAGGTGGTTACTATTTGTGTGAATTCAAATCTACTTACAAGGCAAAGAAGCCTGTGAGGATGCCAGGGTATCACTATGTTGACCGCAAACTGGATGTAACCAGTCACAACAAGGATTACACATTTGTTGAGCAGTGTGAAATATCCATTGCACGCCACTCTTTGCTCGGTCATCACCATCACCATCACTAAAATCCCGCGGCCATGGCGGCCGGGAGCATGCGACGTCGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAANAGGCCCGCACCGATCGCCCTTCCCACAGTTGCGCAGCCTGATGGCNATGGACGCNNCCTGNANCGNCGCATTAGCNCGGCNGGNNNGNGNNCNCGCAGCGNGACNCTACANTTNCNGCNNCNANC

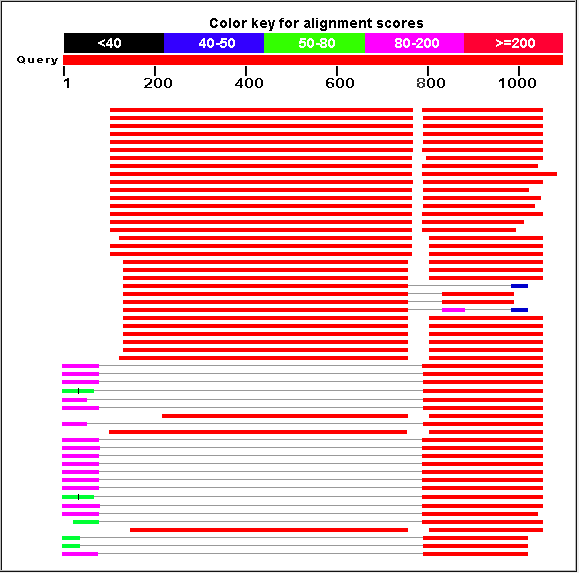
Bases: 1,092

**Result of Blastn of sequencing results with that of Human Genome:**



As we can see no sequences were really able to line up very well. Most ended up with a “blue” score which is pretty low and the highest score was a “green” score which is still considerably low. This score was reached by a Homo sapiens chromosome X, alternate assembly HuRef.

**Result of Blastn of sequencing results with the whole nucleotide collection:**

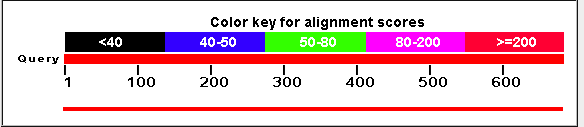


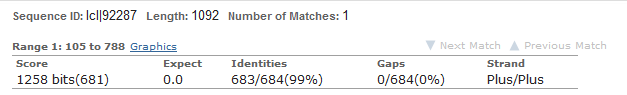
As we can see here there are some very high scores, particularly the solid red bars indicating a very good alignment score. The top alignment score was reached by Montipora efflorescens GFP-like chromoprotein mRNA, complete cds, which happens to actually be the organism and the gene we were trying to sequence.

**Known sequence of the pGBR22:**

ATGAGTGTGATCGCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAATGGACACTACTTTGAGGTCGAAGGCGATGGAAAAGGAAAGCCTTACGAGGGGGAGCAGACGGTAAAGCTCACTGTCACCAAGGGTGGACCTCTGCCATTTGCTTGGGATATTTTATCACCACTGTCTCAATACGGAAGCATACCATTCACCAAGTACCCTGAAGACATCCCTGATTATGTAAAGCAGTCATTCCCTGAGGGATATACATGGGAGAGGATCATGAACTTTGAAGATGGTGCAGTGTGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTACAATGTCAAAATCTCTGGTGTGAACTTTCCTCCCAATGGACCTGTTATGCAGAAGAAGACACAGGGCTGGGAACCCAACACTGAGCGTCTCTTTGCACGAGATGGAATGCTGATAGGAAACAACTTTATGGCTCTGAAGTTGGAAGGAGGTGGTTACTATTTGTGTGAGTTCAAATCTACTTACAAGGCAAAGAAGCCTGTGAGGATGCCAGGGTATCACTATGTTGACCGCAAACTGGATGTAACCAGTCACAACAAGGATTACACATTTGTTGAGCAGTGTGAAATATCCATTGCACGCCACTCTTTGCTCGGTCATCACCATCACCATCACTAA

**Results of Blast2Seq of Known pGBR22 sequence with DNA sequencing results**





Known 1 ATGAGTGTGATCGCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAATGGA 60

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 105 ATGAGTGTGATCGCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAATGGA 164

Known 61 CACTACTTTGAGGTCGAAGGCGATGGAAAAGGAAAGCCTTACGAGGGGGAGCAGACGGTA 120

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 165 CACTACTTTGAGGTCGAAGGCGATGGAAAAGGAAAGCCTTACGAGGGGGAGCAGACGGTA 224

Known 121 AAGCTCACTGTCACCAAGGGTGGACCTCTGCCATTTGCTTGGGATATTTTATCACCACTG 180

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 225 AAGCTCACTGTCACCAAGGGTGGACCTCTGCCATTTGCTTGGGATATTTTATCACCACTG 284

Known 181 TCTCAATACGGAAGCATACCATTCACCAAGTACCCTGAAGACATCCCTGATTATGTAAAG 240

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 285 TCTCAATACGGAAGCATACCATTCACCAAGTACCCTGAAGACATCCCTGATTATGTAAAG 344

Known 241 CAGTCATTCCCTGAGGGATATACATGGGAGAGGATCATGAACTTTGAAGATGGTGCAGTG 300

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 345 CAGTCATTCCCTGAGGGATATACATGGGAGAGGATCATGAACTTTGAAGATGGTGCAGTG 404

Known 301 TGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTACAATGTCAAAATC 360

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 405 TGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTACAATGTCAAAATC 464

Known 361 TCTGGTGTGAACTTTCCTCCCAATGGACCTGTTATGCAGAAGAAGACACAGGGCTGGGAA 420

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 465 TCTGGTGTGAACTTTCCTCCCAATGGACCTGTTATGCAGAAGAAGACACAGGGCTGGGAA 524

Known 421 CCCAACACTGAGCGTCTCTTTGCACGAGATGGAATGCTGATAGGAAACAACTTTATGGCT 480

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 525 CCCAACACTGAGCGTCTCTTTGCACGAGATGGAATGCTGATAGGAAACAACTTTATGGCT 584

Known 481 CTGAAGTTGGAAGGAGGTGGTTACTATTTGTGTGAGTTCAAATCTACTTACAAGGCAAAG 540

||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||

Reslt 585 CTGAAGTTGGAAGGAGGTGGTTACTATTTGTGTGAATTCAAATCTACTTACAAGGCAAAG 644

Known 541 AAGCCTGTGAGGATGCCAGGGTATCACTATGTTGACCGCAAACTGGATGTAACCAGTCAC 600

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 645 AAGCCTGTGAGGATGCCAGGGTATCACTATGTTGACCGCAAACTGGATGTAACCAGTCAC 704

Known 601 AACAAGGATTACACATTTGTTGAGCAGTGTGAAATATCCATTGCACGCCACTCTTTGCTC 660

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Reslt 705 AACAAGGATTACACATTTGTTGAGCAGTGTGAAATATCCATTGCACGCCACTCTTTGCTC 764

Known 661 GGTCATCACCATCACCATCACTAA 684

||||||||||||||||||||||||

Reslt 765 GGTCATCACCATCACCATCACTAA 788

There was one inconsistent base pair found that has been highlighted. This inconsistency may not matter if it still translates into the same amino acid.

**Result of DNA translation using Reading Frame 1:**

YXILKLCIQRVGSSPIWSTCRRPH\*\*F\*LIEGEIS\*V\*SLNK\*PTRFICQARSMDTTLRS

KAMEKESLTRGSRR\*SSLSPRVDLCHLLGIFYHHCLNTEAYHSPSTLKTSLIM\*SSHSLR

DIHGRGS\*TLKMVQCVLSAMIPASKATVSSTMSKSLV\*TFLPMDLLCRRRHRAGNPTLSV

SLHEMEC\*\*ETTLWL\*SWKEVVTICVNSNLLTRQRSL\*GCQGITMLTANWM\*PVTTRITH

LLSSVKYPLHATLCSVITITITKIPRPWRPGACDVGPNSPYSESYYNSLAVVLQRRDWEN

PGVTQLNRLAAHPPFASWRNSEXARTDRPSHSCAA\*WXWTXPXXXH\*XGXXXXXQRDXTX

XAXX

**Result of DNA translation using Reading Frame 2:**

XXYSSYASNALGALPYGRPAGGRTSDFD\*LKEKYHECDR\*TNDLQGLYVRHGQWTLL\*GR

RRWKRKALRGGADGKAHCHQGWTSAICLGYFITTVSIRKHTIHQVP\*RHP\*LCKAVIP\*G

IYMGEDHEL\*RWCSVYCQQ\*FQHPRQLFHLQCQNLWCELSSQWTCYAEEDTGLGTQH\*AS

LCTRWNADRKQLYGSEVGRRWLLFV\*IQIYLQGKEACEDARVSLC\*PQTGCNQSQQGLHI

C\*AV\*NIHCTPLFARSSPSPSLKSRGHGGREHATSGPIRPIVSRITIHWPSFYNVVTGKT

LALPNLIALQHIPLSPAGVIAXRPAPIALPTVAQPDGXGRXLXRRISXAGXXXRSXTLXX

XXX

**Result of DNA translation using Reading Frame 3:**

XXTQAMHPTRWELSHMVDLQAAALVILID\*RRNIMSVIAKQMTYKVYMSGTVNGHYFEVE

GDGKGKPYEGEQTVKLTVTKGGPLPFAWDILSPLSQYGSIPFTKYPEDIPDYVKQSFPEG

YTWERIMNFEDGAVCTVSNDSSIQGNCFIYNVKISGVNFPPNGPVMQKKTQGWEPNTERL

FARDGMLIGNNFMALKLEGGGYYLCEFKSTYKAKKPVRMPGYHYVDRKLDVTSHNKDYTF

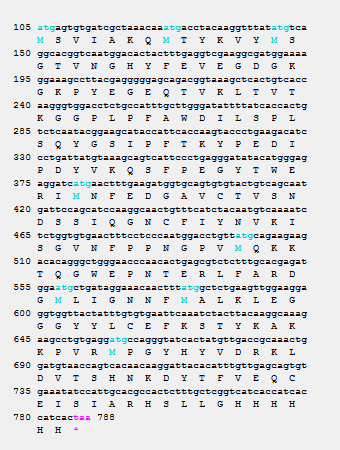
VEQCEISIARHSLLGHHHHHH\*NPAAMAAGSMRRRAQFAL\*\*VVLQFTGRRFTTS\*LGKP

WRYPT\*SPCSTSPFRQLA\*\*RXGPHRSPFPQLRSLMAMDAXXXXALARXXXXXAAXXYXX

XXX

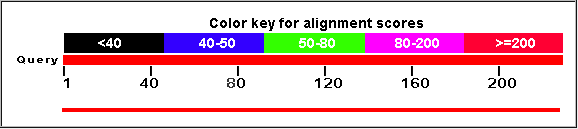
This sequence seems to be the correct sequence because it has the 6-Histidine tag present in the C terminal of the protein.

**ORF Finder results of DNA sequencing results:**



This ORF finder helped determine which Methionine is the start codon of this plasmid. This makes sense because it is the first Methionine after the stop codon found earlier in the sequence and it has a long continuous coding region that goes until the stop codon right after the Histidine tag.

**Results of Blastp for Known pGBR22 amino acid sequence compared to Sequencing Results:**



We can see that there is a solid red bar that is a 100% match for the amino acid sequence.



Known 1 MSVIAKQMTYKVYMSGTVNGHYFEVEGDGKGKPYEGEQTVKLTVTKGGPLPFAWDILSPL 60

MSVIAKQMTYKVYMSGTVNGHYFEVEGDGKGKPYEGEQTVKLTVTKGGPLPFAWDILSPL

Reslt 1 MSVIAKQMTYKVYMSGTVNGHYFEVEGDGKGKPYEGEQTVKLTVTKGGPLPFAWDILSPL 60

Known 61 SQYGSIPFTKYPEDIPDYVKQSFPEGYTWERIMNFEDGAVCTVSNDSSIQGNCFIYNVKI 120

SQYGSIPFTKYPEDIPDYVKQSFPEGYTWERIMNFEDGAVCTVSNDSSIQGNCFIYNVKI

Reslt 61 SQYGSIPFTKYPEDIPDYVKQSFPEGYTWERIMNFEDGAVCTVSNDSSIQGNCFIYNVKI 120

Known 121 SGVNFPPNGPVMQKKTQGWEPNTERLFARDGMLIGNNFMALKLEGGGYYLCEFKSTYKAK 180

SGVNFPPNGPVMQKKTQGWEPNTERLFARDGMLIGNNFMALKLEGGGYYLCEFKSTYKAK

Reslt 121 SGVNFPPNGPVMQKKTQGWEPNTERLFARDGMLIGNNFMALKLEGGGYYLCEFKSTYKAK 180

Known 181 KPVRMPGYHYVDRKLDVTSHNKDYTFVEQCEISIARHSLLGHHHHHH 227

KPVRMPGYHYVDRKLDVTSHNKDYTFVEQCEISIARHSLLGHHHHHH

Reslt 181 KPVRMPGYHYVDRKLDVTSHNKDYTFVEQCEISIARHSLLGHHHHHH 227

We can see here that all of the amino acids lined up with the known amino acid sequence. This tells us that one inconsistency we saw earlier is not a problem because it translates into the same amino acid.

**pGEMT Vector Backbone Sequence:**

GGGCGAATTGGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCCGCGGGATTAATCACTAGTGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATA

Flanking Regions are highlighted in pink and gray.

**pGEMT Vector Backbone Reverse Complement Sequence:**

TATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATCCAACGCGTTGGGAGCTCTCCCATATGGTCGACCTGCAGGCGGCCGCACTAGTGATTAATCCCGCGGCCATGGCGGCCGGGAGCATGCGACGTCGGGCCCAATTCGCCC

Flanking Regions are highlighted in pink and gray.

**M13 Original DNA Sequence Result:**

TATNGNATACTCAAGCTATGCATCCAACGCGTTGGGAGCTCTCCCATATGGTCGACCTGCAGGCGGCCGCACTAGTGATTTTGATTGATTGAAGGAGAAATATCATGAGTGTGATCGCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAATGGACACTACTTTGAGGTCGAAGGCGATGGAAAAGGAAAGCCTTACGAGGGGGAGCAGACGGTAAAGCTCACTGTCACCAAGGGTGGACCTCTGCCATTTGCTTGGGATATTTTATCACCACTGTCTCAATACGGAAGCATACCATTCACCAAGTACCCTGAAGACATCCCTGATTATGTAAAGCAGTCATTCCCTGAGGGATATACATGGGAGAGGATCATGAACTTTGAAGATGGTGCAGTGTGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTACAATGTCAAAATCTCTGGTGTGAACTTTCCTCCCAATGGACCTGTTATGCAGAAGAAGACACAGGGCTGGGAACCCAACACTGAGCGTCTCTTTGCACGAGATGGAATGCTGATAGGAAACAACTTTATGGCTCTGAAGTTGGAAGGAGGTGGTTACTATTTGTGTGAATTCAAATCTACTTACAAGGCAAAGAAGCCTGTGAGGATGCCAGGGTATCACTATGTTGACCGCAAACTGGATGTAACCAGTCACAACAAGGATTACACATTTGTTGAGCAGTGTGAAATATCCATTGCACGCCACTCTTTGCTCGGTCATCACCATCACCATCACTAAAATCCCGCGGCCATGGCGGCCGGGAGCATGCGACGTCGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAANAGGCCCGCACCGATCGCCCTTCCCACAGTTGCGCAGCCTGATGGCNATGGACGCNNCCTGNANCGNCGCATTAGCNCGGCNGGNNNGNGNNCNCGCAGCGNGACNCTACANTTNCNGCNNCNANC

Flanking Regions are highlighted in pink and gray. The sequence between these regions will be inserted in the vector backbone.

**Sequence of pGBR22:**

TATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACA**CAGGAAACAGCTATGAC**CATGATTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTATGCATCCAACGCGTTGGGAGCTCTCCCATATGGTCGACCTGCAGGCGGCCGCACTAGTGATTTTGATTGATTGAAGGAGAAATATCATGAGTGTGATCGCTAAACAAATGACCTACAAGGTTTATATGTCAGGCACGGTCAATGGACACTACTTTGAGGTCGAAGGCGATGGAAAAGGAAAGCCTTACGAGGGGGAGCAGACGGTAAAGCTCACTGTCACCAAGGGTGGACCTCTGCCATTTGCTTGGGATATTTTATCACCACTGTCTCAATACGGAAGCATACCATTCACCAAGTACCCTGAAGACATCCCTGATTATGTAAAGCAGTCATTCCCTGAGGGATATACATGGGAGAGGATCATGAACTTTGAAGATGGTGCAGTGTGTACTGTCAGCAATGATTCCAGCATCCAAGGCAACTGTTTCATCTACAATGTCAAAATCTCTGGTGTGAACTTTCCTCCCAATGGACCTGTTATGCAGAAGAAGACACAGGGCTGGGAACCCAACACTGAGCGTCTCTTTGCACGAGATGGAATGCTGATAGGAAACAACTTTATGGCTCTGAAGTTGGAAGGAGGTGGTTACTATTTGTGTGAATTCAAATCTACTTACAAGGCAAAGAAGCCTGTGAGGATGCCAGGGTATCACTATGTTGACCGCAAACTGGATGTAACCAGTCACAACAAGGATTACACATTTGTTGAGCAGTGTGAAATATCCATTGCACGCCACTCTTTGCTCGGTCATCACCATCACCATCACTAAAATCCCGCGGCCATGGCGGCCGGGAGCATGCGACGTCGGGCCCAATTCGCCC

Flanking regions are highlighted in pink and gray.

Shine-Dalgarno Sequence is highlighted in cyan.

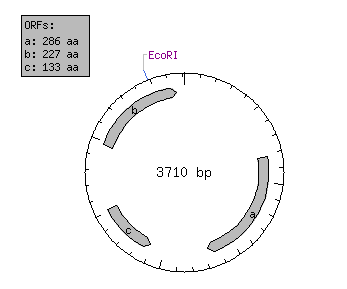
Start codon is highlighted in green.

Stop codon is highlighted in red.

M13 Rev sequence is underlined.

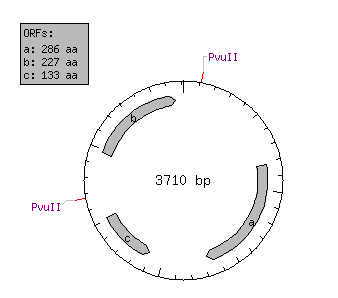
M13 Rev primer site is underlined and bold.

**Restriction Enzyme Digest Using EcoRI:**



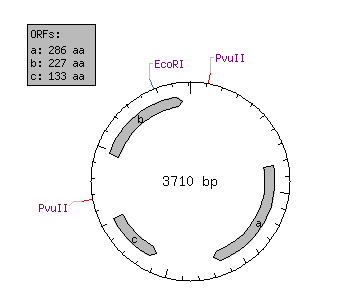
This is a one-cutter enzyme. EcoRI can only be run with the NEBuffer 2.1

**Restriction Enzyme Digest Using PvuII:**



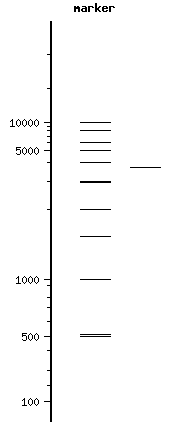
This is a two-cutter enzyme so it will cut the plasmid in two places. PvuII can be run with Cutsmart Buffer, NEBuffer 2.1, or NEBuffer 3.1.

**Restriction Enzyme Digest Using EcoRI+PvuII:**

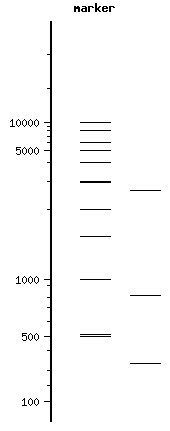


This will cut the plasmid in three places. Both these enzymes can be run using NEBuffer 2.1 so yes they can be run together in the same reaction tube. None of these enzymes are methylation sensitive.

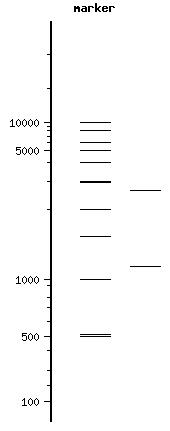
**Restriction Enzyme Digest of pGBR22 with EcoRI**



**Restriction Enzyme Digest of pGBR22 with PvuII and EcoRI**



**Restriction Enzyme Digest of pGBR22 with PvuII**



You can determine the sequence of a target sequence bigger than 1000 bp by using restriction enzymes to cut it into chunks that are smaller than 1000 bp, then doing 3 PCR’s to amplify each individual chunk. You can then send each of those samples to DNA sequencing and analyze the results to form your entire sequence. You can also do shotgun sequencing where you overlap individual pieces of DNA to form the entire sequence.